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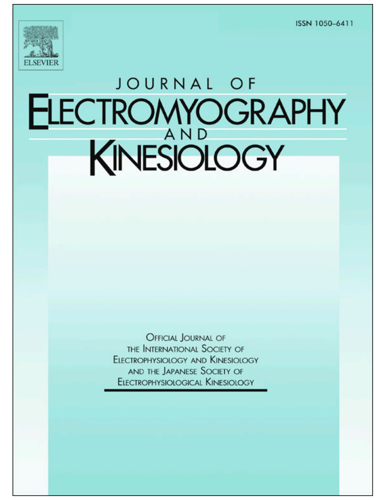

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Chiropractic spinal manipulation alters TMS induced I-wave excitability and shortens the cortical silent period

Heidi Haavik[1], Imran Khan Niazi [1,2,3], Mads Jochumsen[2], Paulius Uginčius[4], Oğuz Sebik[4], Gizem Yılmaz [4], Muhammad Samran Navid [1,2], Mustafa Görkem Özyurt [4], Kemal S. Türker[4]

[1]Centre for Chiropractic Research, New Zealand College of Chiropractic, Auckland, New Zealand.

[2] SMI, Department of Health Science and Technology, Aalborg University, Denmark.

[3]Health & Rehabilitation Research Institute, Auckland University of Technology, Auckland, New Zealand.

[4]School of Medicine, Koç University, Istanbul, Turkey

Corresponding author:

Imran Khan Niazi, PhD

Centre for Chiropractic Research,

New Zealand College of Chiropractic,

6 Harrison Road,

Mount Wellington, 1060

Auckland, New Zealand.

imran.niazi@nzchiro.co.nz

ABSTRACT

The objective of this study was to construct peristimulus time histogram (PSTH) and peristimulus frequencygram (PSF) using single motor unit recordings to further characterize the previously documented immediate sensorimotor effects of spinal manipulation. Single pulse transcranial magnetic stimulation (TMS) via a double cone coil over the tibialis anterior (TA) motor area during weak isometric dorsiflexion of the foot was used on two different days in random order; pre/post spinal manipulation (in eighteen subjects) and pre/post a control (in twelve subjects) condition. TA electromyography (EMG) was recorded with surface and intramuscular fine wire electrodes. Three subjects also received sham double cone coil TMS pre and post a spinal manipulation intervention. From the averaged surface EMG data cortical silent periods (CSP) were constructed and analysed. Twenty-one single motor units were identified for the spinal manipulation intervention and twelve single motor units were identified for the control intervention. Following spinal manipulations there was a shortening of the silent period and an increase in the single unit I-wave amplitude. No changes were observed following the control condition. The results provide evidence that spinal manipulation reduces the TMS-induced cortical silent period and increases low threshold motoneurone excitability in the lower limb muscle. These finding may have important clinical implications as they provide support that spinal manipulation can be used to strengthen muscles. This could be followed up on populations that have reduced muscle strength, such as stroke victims.

Key words: Chiropractic, Spinal manipulation; Sensorimotor Integration; Transcranial Magnetic Stimulation; Motor Cortex; Single motor unit; Cortical silent period.

INTRODUCTION

Spinal manipulation has been reported to help individuals suffering from neck pain [Bronfort et al., 2012, 2004], back pain [Bronfort et al., 2004; Kuczynski et al., 2012], and headaches [Gross et al., 2010]. Spinal manipulation has also been shown to improve other aspects of nervous system function, such as improved elbow joint position sense and altered feedforward activation of core abdominal muscles [Haavik and Murphy, 2011; Marshall and Murphy, 2006]. Several studies have found evidence that suggest spinal dysfunction may lead to altered afferent input to the CNS from deep paraspinal muscle afferents [Bolton and Holland, 1998, 1996; Murphy et al., 1995; Zhu et al., 2000, 1993]. This has led to the hypothesis that such altered afferent input may lead to maladaptive central neural plastic changes in somatosensory processing and motor control of not only spinal muscles but also of limb muscles [Haavik-Taylor and Murphy, 2007a]. This altered afferent input from dysfunctional spinal segments is thought to alter the brain's inner body schema because it has been shown that even a history of mild recurrent neck dysfunction alters cerebellar-M1 processing for an upper limb muscle [Daligadu et al., 2013], and spinal manipulation has been shown to reverse this back to what is seen in healthy populations [Daligadu et al., 2013]. These findings support the theory that spinal manipulation, aimed at improving the movement patters of dysfunctional segments thus altering the afferent input from them to the CNS, should be able to reverse this effect [Haavik-Taylor and Murphy, 2007a].

Several studies have shown altered central processing following spinal manipulation by recording somatosensory evoked potentials (SEP) [Haavik-Taylor and Murphy, 2007a, 2007b]. The changes were only observed at the level of the cortex, with altered N20 and N30 SEP peak amplitudes [Haavik-Taylor and Murphy, 2007a, 2007b]. The N20 SEP peak is known to be generated in the primary somatosensory cortex (S1), while the N30 SEP peak is thought to reflect sensorimotor integration [Rossi et al., 2003], in a more complex cortical and subcortical loop linking the basal ganglia, thalamus, pre-motor areas, and primary motor cortex [Kanovsky et al., 2003; Mauguiere et al., 1983; Rossini et al., 1989, 1987; Waberski et al., 1999].

Two studies have shown changes in upper limb motor control following cervical manipulation by finding a significant shortening of the transcranial magnetic stimulation (TMS)-induced cortical silent period (CSP) [Haavik-Taylor and Murphy, 2007b; Taylor and Murphy, 2008]. Both studies have shown a shortening of the CSP with no changes in MEP amplitude [Haavik-Taylor and Murphy, 2007b; Taylor and Murphy, 2008]. However, Türker and colleagues have utilized a different method by constructing peristimulus frequencygram (PSF) [Türker and Cheng, 1994; Türker and Powers, 2005] from single motor unit recordings to further characterize these CSP responses evoked by TMS [Todd et al., 2012]. They found that using a combination of probability and frequency-based analysis techniques to characterize the TMS-induced CSP observed following both subthreshold and suprathreshold TMS during a weak contraction resulted in longer silent periods than previously reported in the literature. Their study highlights the importance of using both probability and frequency-based analysis to accurately determine the duration of inhibitory events in peripheral recordings. Due to this evidence, the aim of the current study was to re-investigate the previous changes in CSP observed following spinal manipulation in lower limb using single motor unit data and a combination of surface EMG (SEMG), peristimulus time histogram (PSTH) and PSF analyses on tibialis anterior (TA).

METHODS

Subjects

In total nineteen subjects participated in one or more experimental sessions. They were sixteen males and three females aged 23 to 39 (mean age 29.4 ± 5.7 years). Subjects were randomly allocated to either the spinal manipulation or control intervention. Experimental measures were then recorded before and after either the spinal manipulation intervention (n = 18) or the control intervention (n = 12). The two experimental days for each subject were at least one week apart. To be included, subjects could not have a history of neurological disease, or any known contraindications to either spinal manipulation or magnetic stimulation (described in more detail below). The subjects were furthermore required to have a history of reoccurring spinal pain or stiffness (e.g. present during the performance of certain tasks such as work or study). However, at the time of the experiment all subjects were required to be pain free. This was done in order to assess the potential effects of spinal manipulation delivered to dysfunctional joints alone without the presence of acute pain, as the presence of pain is known to alter corticomotor measures [Cheong et al., 2003; Eisenberg et al., 2005; Kofler et al., 2001]. Koç University Human Ethical Committee approved the study in accordance with the Declaration of Helsinki. Written informed consent was obtained prior to participation.

Transcranial magnetic stimulation (TMS)

A MagStim 200 (MagStim, Dyfed UK) magnetic stimulators and a double cone coil was used to deliver single pulse magnetic stimuli over the motor cortical area optimal for eliciting MEPs from the TA. This optimal site was then marked on the scalp to ensure identical placement of the coil throughout the experiment. Resting and active motor threshold were determined. Resting motor threshold (RTh) was defined as the minimal stimulus intensity at which five out of ten consecutive stimuli evoked a MEP with amplitude of at least 50 μV in the TA muscle at rest. The active motor threshold (ATh) was defined as the minimal stimulus intensity at which five out of ten consecutive stimuli evoked a MEP with an amplitude of at least 100μV in TA muscle while holding a weak isometric background contraction (about 10% maximum voluntary contraction, MVC).

Electromyographic recording

Surface EMG activity was recorded from the left TA muscle. Two surface electrodes (2cm apart) were placed over TA. The ground electrode was placed on the lateral malleoli at the distal end of the fibula. EMG signals were collected from 7 mm diameter Hydrospot Ag/AgCl electrodes (Physiometrix Inc., USA), fixed with tape following standard skin preparation to reduce electrode impedance to less than 5 kΩ. EMG signals were amplified by a custom-built EMG amplifier, and were recorded from a CED Power 1401 mk 2 data acquisition board and band-pass filtered at 20 Hz – 1 kHz, sampled at a rate of 2 KHz by a Spike2 data acquisition system (CED, UK) and stored on disk for off-line analysis.

For the intramuscular recordings, Teflon insulated silver fine wire bipolar electrodes were inserted into the muscle using a 25-gauge needle that was immediately withdrawn leaving the fish-hooked wires in the muscle. EMG signals recorded from wire electrodes were amplified (x 1000), filtered (100–5000 Hz bandwidth) and sampled (10 kHz) for later analysis using the data acquisition system (details are given above). Single motor unit potentials were identified from the fine wire recordings with the use of Spike2 algorithms. Several hundred responses to the magnetic stimuli were recorded to enable CUSUMs to be calculated from the PSF to pinpoint the subtle changes in discharge rate that are not visible to the eye (details of the technique are published in [Todd et al., 2012]).

Motor evoked potentials (MEPs) and cortical silent period (CSP) duration

MEP latency was established using suprathreshold stimulus intensity and determining the time for the first significant deflection away from the stimulus time. The MEPs and CSPs of the SEMG and units were recorded while the subject contracted the muscle to regularly discharge an identifiable unit in the intramuscular electrodes. The magnetic stimulus was set to an intensity that generated a discharge of the unit at the MEP latency 3 times out of 10 single TMS stimuli. This makes the strength of the excitatory post synaptic potential (EPSP) about 3mV according to the estimation worked out by [Miles et al., 1989]. This stimulus intensity was kept the same pre vs post either intervention.

Interventions

Spinal Manipulation

The entire spine and sacroiliac joints were assessed for segmental dysfunction (also known as vertebral subluxation by the chiropractic profession) and adjusted where deemed necessary by a registered chiropractor with at least ten years clinical experience. The clinical indicators that were used to assess the function of the spine prior to and after each spinal manipulation intervention included assessing for tenderness to palpation of the relevant joints, manually palpating for restricted intersegmental range of motion, assessing for palpable asymmetric intervertebral muscle tension, and any abnormal or blocked joint play and end-feel of the joints. For this study a segment was defined as dysfunctional if at least three of these indicators were present at the same segmental level. All of these biomechanical characteristics are known clinical indicators of spinal dysfunction [Cooperstein et al., 2013, 2010; Fryer et al., 2004; Hestbaek and Leboeuf-Yde, 2000; Hubka and Phelan, 1994; Jull et al., 1988]. All the spinal manipulations carried out in this study were high-velocity, low-amplitude thrusts to the spine or pelvic joints and took about 15 minutes to perform. This is a standard manipulation technique used by chiropractors. The mechanical properties of this type of CNS perturbation have been investigated; and although the actual force applied to the subject's spine depends on the therapist, the patient, and the spinal location of the manipulation, the general shape of the force-time history of spinal manipulations is very consistent [BW et al., 1990] and the duration of the thrust is always less than 200 milliseconds (for review see [Herzog, 1996]). The high-velocity type of manipulation was chosen specifically because previous research [Herzog et al., 1995] has shown that reflex EMG activation observed after manipulations only occurred after high-velocity, low-amplitude manipulations (as compared with lower-velocity mobilizations). This manipulation technique has also been previously used in studies that have investigated neurophysiological effects of spinal manipulation, as discussed in the Introduction (for review see [Haavik and Murphy, 2012]). The subjects received manipulations only to joints that were deemed to be dysfunctional as described above. Each subject received on average 3-5 manipulations, and most were manipulated in all three regions of the spine (cervical, thoracic and lumbar/pelvic regions).

Control intervention

The control intervention consisted of passive and active movements of the subject’s head, spine and body that was carried out by the same chiropractor who pre-checked the subjects for vertebral subluxations and who performs the spinal manipulations in the experimental intervention session. This control intervention involved the subjects being moved into the manipulation setup positions where the chiropractor would normally apply a thrust to the spine to achieve the manipulations. However, the experimenter was particularly careful not to put pressure on any individual spinal segments. Loading a joint, as is done prior to spinal manipulation has been shown to alter paraspinal proprioceptive firing in anesthetised cats [Pickar and Wheeler, 2001], and was therefore carefully avoided by ending the movement prior to end-range-of-motion when passively moving the subjects. No spinal manipulation was performed during any control intervention. This control intervention was not intended to act as a sham manipulation but to act as a physiological control for possible changes occurring due to the cutaneous, muscular or vestibular input that occur with the type of passive and active movements involved in preparing a subject/patient for a manipulation. It also acted as a control for the effects of the stimulation necessary to collect the dependent measures of the study, and acted as a control for the time required to carry out the manipulation intervention.

Experimental protocol

Subjects were first given written and verbal information, and signed informed consent was obtained. The subject's spine was then checked by a registered chiropractor to determine if and where the spines would be manipulated as described above. If the subject was judged to have segmental dysfunction, the relevant information (including detailed medical history) was then obtained. Subjects were screened for contraindications for manipulation, such as recent history of trauma, known conditions such as inflammatory or infectious arthropathies and bone malignancies. Finally, subjects were screened for contraindications for magnetic stimulation, such as a history of epilepsy, pregnancy, or metal implants in the head.

Experimental measures were then recorded before and after either the control intervention (n = 13) or the spinal manipulation intervention (n = 17). The two experimental days for each subject were at least one week apart and the order was randomised. Both interventions took about 15 minutes to perform, as described above. Subjects began the first experiment with maximal voluntary dorsiflexions of the foot. Three brief (2–3 s) isometric contractions were performed and each contraction was separated by greater than 1 min of rest to avoid fatigue. Active motor threshold was then measured during weak foot dorsiflexion (~10% MVC) and rest threshold was measured during no contraction. Stimuli were delivered at a frequency of 0.25–0.33 Hz and initially at a clearly suprathreshold level. The stimulus intensity was then reduced in small increments until the intensity was at motor threshold. Subjects maintained the weak contraction to activate a clearly distinguishable motor unit at a regular rate (details are given above and published elsewhere: [Todd et al., 2012]). Visual and auditory feedback of single motor unit discharge was provided. Subjects then rested for several minutes and repeated the sustained weak foot dorsiflexion until up to a few hundred TMS stimuli was delivered so that we can induce reliable PSTHs and PSFs.

Three subjects repeated the protocol on a different day with sham TMS. Sham TMS was applied by holding the coil perpendicular to the skull so the magnetic stimuli would be applied into the air in front of the subject. The subject would therefore felt the coil over the head and hear the same sounds, but does not receive any stimulation [Loo et al., 2000]. Sham stimulation was applied to determine if other factors, such as the auditory click that accompanies discharge of the Magstim capacitor, contribute to suppression of voluntary EMG observed after real threshold stimulation.

Data analysis

For brief maximal contractions, root mean square (RMS) SEMG was measured over a 1 s interval. Pre-stimulus voluntary RMS SEMG was normalized to the average obtained during brief maximal efforts. For the sustained weak contraction during the activity of the motor unit, SEMG analysis involved extraction of a defined time period from around the stimuli (± 250 ms) averaging the signals and normalizing the data to the MVC of the muscle. Cumulative sum (CUSUM) [Ellaway, 1978] was calculated from the averaged SEMG to illustrate the timing of the response in the SEMG.

Analysis of SEMG included the peak-to-peak amplitude and latency (onset) of MEPs measured from the unrectified SEMG. The duration of the CSP following TMS was measured by a cursor from the stimulus onset to the end of the significant downturn in the rectified SEMG CUSUM [Brinkworth and Türker, 2003].

Analysis of the electrical activity recorded with intramuscular fine wire electrodes involved identification of single motor unit potentials followed by construction of PSTH and PSF. While PSTH is a simple histogram indicating the timing of spikes against the stimulus, PSF is made up of superimposition of the instantaneous discharge rates of a selected unit around the time of the stimulus and indicates the changes in the membrane potential of the motoneurone [Türker and Cheng, 1994; Türker and Powers, 2005]. To build PSF and PSTH, electrical activity from the intramuscular fine wire electrodes was displayed and the shape of one large individual motor unit action potential (spike) was defined as a template in the Spike2 program. During the experiment and during off-line analysis, any spike whose shape matched this pre-established template generated acceptance pulses in the program. The acceptance pulses from the discriminated units were then used to construct PSTHs and PSFs around the time of stimulation. PSTH and PSF CUSUMs were then constructed from data normalised to the average prestimulus values. PSTH and PSF responses were compared with SEMG responses. CUSUMs for the PSTH graphs were also obtained to make any subtle but significant changes in bin counts detectable. Similarly, CUSUMs for the PSF were calculated to pinpoint the subtle changes in discharge rate that were not visible to the eye (details of the technique are published in [Todd et al., 2012]).

In line with our previous studies [Brinkworth and Türker, 2003; Türker et al., 1997], we only considered significant changes in the post-stimulus period that occurred before the minimum reaction time to the stimulus. Minimum reaction time to the TMS stimulation sound on TA EMG was found to be 281±24 ms in the current study. Any post-stimulus changes that occur within this timeframe (i.e. less than 250 ms) are therefore considered not to be contaminated by conscious effort. Any apparent post-stimulus deflection was then considered as a genuine response to the stimulus only if they were larger than the prestimulus error box [Brinkworth and Türker, 2003; Türker et al., 1997] and occurred before the reaction time to the stimulus. If such deflections were going up, they are classified as ‘excitation’ and if they were going down, as ‘inhibition’. Onset of inhibition or excitation was taken as the first apparent deflection that was larger than the error box. The endpoint of inhibition or excitation was the point where the significant deflection ceased and the CUSUM became flat again.

For statistical analysis, two-way ANOVA with factors time (pre and post) and intervention (Spinal manipulation and Control) was applied separately for each of the measured parameters. Statistical significance was assumed if $P<0.05$.

RESULTS

In total 85 individual units from across all 19 subjects were identified. To ensure quality and clarity of the PSTH and PSF records, we set the minimum number of stimuli to be 75, after which we were left with 64 individual units. To investigate changes post intervention, we only accepted units that were identical pre and post intervention using the macro EMG of the unit's reflection on the SEMG, as previously described [Schmied and Türker, 2001]. Thirty-seven units fit the 75 stimuli criteria for the spinal manipulation intervention. Twenty-one of these were identical pre and post spinal manipulation. Twenty-four units fit the 75 stimuli criteria for the control intervention analysis. Twelve of them were identical pre and post the control intervention. Three units were utilized for the sham TMS manipulation intervention. Only one unit was identical pre and post manipulation with the sham TMS stimulation. No changes in active or rest threshold were found after either intervention.

SEMG CUSUM

CSP was measured according to previously published methods (i.e. measured from stimulus onset to reoccurrence of SEMG) [Haavik-Taylor and Murphy, 2007b; Taylor and Murphy, 2008] in the current study, we found a significant interaction effect for intervention and time ($F(1,28)=10.66, p = 0.003$). Post hoc analysis revealed significant ($p = 0.002$) decrease of 19.42 ms in duration of inhibition after spinal manipulation only (See Table 1). No change in CSP duration was observed following the control intervention. Fig 1 illustrates the SEMG results of a subject before and after spinal manipulation. This is an average of 88 stimuli and shows clearly that no change in the MEP amplitude occurs following the manipulation. Table 1 illustrates the CSP results analysed from averaged SEMG data from all subjects, as well as measures from the single motor units (SMUs) that were identical pre and post spinal manipulation and control intervention. All methods of analysis for CSP duration resulted in a significant reduction following spinal manipulation with no change following the control intervention.

Single motor unit results

Prestimulus time histogram (PSTH)
We have used the timing of the stimulus and the acceptance pulses of single motor units as the source to obtain PSTH records. PSTHs and the CUSUMs built upon the PSTHs have shown the following findings: The latency for the MEP was found to be similar to the one found using the SEMG records. The MEPs of single units displayed several peaks separated by a few milliseconds reminiscent to the I-waves described earlier by many investigators (for review see [Awiszus and Feistner, 1994, 1993; Lazzaro and Ziemann, 2013]). The number and the size of these waves changed following the manipulation, which is summarized in Table 2. Twelve identical SMUs were identified, and while average onset latency for the first I-wave was similar for both pre and post manipulation, the amplitude increased post manipulation by 90% on average. Of the eight identical SMUs that were identified that had a second I-wave, again the onset latencies were similar, and again the amplitudes increased post-manipulation by 180% on average. These average increases were not significant due to large variability. Examples are shown in Fig 2 and 3 (pre and post spinal manipulation) and Fig 4 (pre and post control) where the number of I-waves were visually identified. The PSTHs of the I-waves are illustrated in an expanded time scale in Fig 5 and 6.

For the CSP duration significant interaction effect for intervention and time ($F(1,31) = 5.863$, $p = 0.022$). was found for intervention and time when measured using the onset and end points identified from the SMU PSTH CUSUM analysis. Post hoc analysis showed that the CSP duration on average decreased significantly($p=0.001$) by 25.3ms after the manipulation, again in congruence with previous published findings [Haavik-Taylor and Murphy, 2007b; Taylor and Murphy, 2008]. Following the control intervention there was no significant change on the number of I-waves, on the amplitude of the MEPs, or on the CSP duration. In Fig 2 (Panel b) and Fig 3 (Panel b) there is a clear shortening of the CSP in the PSTH CUSUM post manipulation.

Peristimulus Frequencygram (PSF)

Using instantaneous discharge rates of single motor units the latency of the MEP was the same as the PSTH records (see Table 3). The MEP was made up of several temporally separated excitatory post synaptic potentials as indicated by genuine increases of the discharge rates underlying the PSTH peaks (See Fig 2, 3 and 4). In some units, although the PSTH indicated CSPs as the lower number of action potential occurrences, the PSF failed to indicate any significant discharge rate reduction underlying those periods, hence suggesting that they may be false periods of inhibitions. For example, Fig 3 - first column - panel B, there is what appears to be an inhibition in the PSTH CUSUM. However, there is no change to the PSF CUSUM in panel D indicating that the discharge rate did not decrease, hence no genuine inhibition during CSP.

Following spinal manipulation, in the SMUs that displayed true excitation in the PSF CUSUM (n=12), we observed a significant increase ($p = 0.01$) in I-wave amplitude for the first I-wave peak. The second I-wave also increased, however, this increase was not significant. Individual examples of these increases can be observed in Fig 2 and 3, panel d. In two subjects (four identical pairs of SMUs pre-vs post manipulation) it was noted that there was a true excitation in the PSF CUSUM prior to the spinal manipulations, yet after the intervention these identical units were no longer showing any excitation with the magnetic stimulation.

In subjects showing genuine inhibition in the PSF (7 SMUs pre/post SM and 5 SMUs pre/post control) the CSP was measured from beginning point taken from PSTH and end point from PSF, as recommended by [Todd et al., 2012]. Two way revealed significant interaction effect for intervention and time ($F (1,10) =7.35$, $p = 0.02$). Post hoc analysis showed that the CSP decreased significantly post manipulation ($p =0.02$) with an average decrease in duration of 34.7ms.

Background SMU firing rate

Analysing the background discharge rates from the identical units pre vs. post intervention (measured pre-stimulus onset) there were no significant changes in firing rate following the spinal manipulation or control interventions.

DISCUSSION

This study has discovered three novel findings: Firstly, this study demonstrated in human subjects the existence of individual I-waves using single motor unit recordings. The I-waves were observed in the single unit data as separate entities with significant peaks and are confirmed to be *excitatory* events as the discharge rate underlying them were higher than the background rate. Secondly, chiropractic manipulation significantly increased the *amplitude of the first I-wave*. Finally, the *CSP duration* was significantly reduced after spinal manipulation in lower limb muscle.

I-waves

Transcranial magnetic stimulation can activate the human brain through the intact scalp [Barker et al., 1985; Merton and Morton, 1980]. A single TMS pulse evokes a series of descending corticospinal volleys that are separated from each other by about 1.5ms (for review see [Lazzaro and Ziemann, 2013]). The evoked descending corticospinal activity has been directly recorded from epidural electrodes placed over the high cervical cord in both animals and human subjects [Lazzaro and Ziemann, 2013]. The earliest wave is thought to originate from the direct activation of the axons or the axon hillock of fast-conducting pyramidal tract neurones (PTN) and is therefore termed the “D” wave [Lazzaro et al., 1999]. The later waves are thought to originate from indirect (i.e. trans-synaptic) activation of PTNs and are therefore termed “I” waves [Patton and Amassian, 1954]. The various I-waves tend to be numbered using their latency from the time of stimulation.

In this study, the individual peaks in the PSTH at the MEP latency that are separated by a few milliseconds, are clearly observed in all units tested and demonstrated in Fig 2 and 3. For the purpose of this paper these peaks at the latency of the MEP are referred to as *I-waves* as they match exactly the timing of the I-waves recorded directly from the spinal cord. We are not the only researchers referring to these PSTH peaks as I-waves as other researchers also suggested that these peaks were indications of the I-waves [Awiszus and Feistner, 1994]. As the sign of these waves, in the current study, using the PSF results we have proven that these I-waves were genuine excitatory events as the discharge rates underlying these peaks were higher than the background firing rates.

It has been previously shown that TMS with the double cone coil for lower limb stimulation at stimulus intensities around motor threshold, preferentially evoked EMG responses that were later than those after anodal stimulation, suggesting I-wave activation at these low intensities [Terao et al., 2000]. It was argued that stimulation of the leg area was fundamentally the same as stimulation of the hand area, in that, magnetic stimuli tended to evoke I-waves, whereas vertex electric stimulation preferentially recruits D-waves. Our findings supports notion of Terao et al. 2000, as the current results also suggest I-wave activation because the time of the MEP onsets are too long to be direct activation of PTNs.

A single motor unit recording protocol has also been described to study the differential activation of corticospinal volleys by various types of transcranial magnetic stimulation (TMS) by [Terao et al., 2001]. Using the protocol in [Terao et al., 2001], the authors were unable to demonstrate selective activation of the various I-waves for the leg motor area, as we have demonstrated in the current study. However, others have shown the I-waves in individual motor units similar to our findings [Awiszus and Feistner, 1994]. This study differs from the previous studies in that we have shown that each one of these I-wave peaks in the PSTH records are genuine excitatory events as the discharge rates underlying these peaks were higher than the prestimulus discharge rates.

Spinal manipulation and increased I-wave excitation

This study demonstrated that a single session of chiropractic spinal manipulation altered the amplitude and the number of the observed I-waves recorded from identical SMUs. This was an unexpected but a significant finding as it proves that the waves can be strengthened or may be joined up to make larger excitations. This needs to be examined further, as this has significant implications for clinical application to a variety of patient populations. The first I-waves increased post manipulation significantly. The changes observed in this study are therefore strong evidence that spinal manipulation can result in a significant increase in excitability of some low threshold motor units. This finding is congruent with the findings of a separate study utilizing the H-reflex methodology [Niazi et al., 2015], where we found a decrease in the H-reflex threshold following spinal manipulation, also suggesting that manipulation selectively alters the excitability of low-threshold motor units, in this case for the soleus muscle.

It is possible that the spinal manipulation intervention also lead to a change in level of intra-cortical or spinal inhibition for the four SMUs that were excited prior to the spinal manipulation intervention yet showed no excitation after the intervention. This suggests a change in the SMU recruitment taking place following spinal manipulation, with some SMUs no longer excited after spinal manipulation while other SMUs are apparently 'switched off'. However, it is possible that despite our careful efforts to stimulate the exact same spot on the scalp before and after our interventions that post manipulation the magnetic stimuli were applied at a slightly different spot or angle and that these particular SMUs were no longer activated by the magnetic stimuli.

Spinal manipulation and the cortical silent period

A significant decrease of the CSP in the low-threshold units was observed in the current study. We know from our earlier experience that the inhibitory synaptic events as expressed indirectly in the regularly active single motor units is highly depend upon the background discharge rate of the unit concerned [Miles and Türker, 1987, 1986]. We have named this phenomenon 'the frequency principle of inhibition' [Miles and Türker, 1986] since the duration of an inhibitory event lasted longer when the motor unit used to study the inhibition fired slower even when the stimulus intensity was fixed. With this knowledge, we expect that the prestimulus discharge rate may play an important role in the expression of the inhibitory synaptic potentials. In our study however the background discharge rate of the motor units remained the same after the SM where the CSP duration was found to be shorter. Therefore, we are confident that the change in the CSP must have been genuine. The current results found that even though the discharge rate of the identical unit remained stable, the inhibitory period actually became shorter. We can therefore conclude with confidence that in the low-threshold units, the chiropractic manipulation session induces genuine reduction in CSP duration.

The CSP has been claimed to reflect both spinal and cortical inhibitory components [Brasil- Neto et al., 1995; Cantello et al., 1992; Chen et al., 1999; Inghilleri et al., 1993; Kukowski and Haug, 1992; Roick et al., 1993]. Studies have shown that the first part of the CSP (about 50ms) after TMS is produced mainly by spinal mechanisms such as after-hyperpolarization and Renshaw recurrent inhibition of the spinal motoneurones [Chen et al., 1999; Inghilleri et al., 1993]. Reciprocal inhibitory effects on the target muscle may also contribute, since the magnetic stimulation often causes simultaneous activation of antagonists. However, the rest of the CSP (after about 50ms) is produced mainly by cortical inhibition [Brasil- Neto et al., 1995; Cantello et al., 1992; Chen et al., 1999; Inghilleri et al., 1993; Kukowski and Haug, 1992; Roick et al., 1993].

The exact mechanisms of the cortical inhibition responsible for producing the CSP are however more difficult to establish. Most evidence suggests that this inhibition is presynaptic to the cortico-spinal neurons, rather than due to a decreased excitability of these cortico-spinal neurons [Cantello et al., 1992; Inghilleri et al., 1993; Tergau et al., 1999]. Neuropharmacological modulation in healthy subjects suggests that the CSP reflects $GABA_B$-mediated intracortical inhibition [Siebner et al., 1998; Werhahn et al., 1999]. Some have argued that it results from activation of inhibitory neurons projecting onto the pyramidal cells of the motor cortex [Inghilleri et al., 1993]. However, it may also reflect a withdrawal of excitatory input to pyramidal cells, by increased inhibition of such excitatory pathways.

The results of the current study have confirmed previous findings that the chiropractic manipulation does not alter the size of the MEP but reduces the duration of the CSP [Haavik-Taylor and Murphy, 2007b; Taylor and Murphy, 2008]. These previous studies showed this to be the case in upper limb muscles. The current study demonstrates that this is also the case for a lower limb muscle. However, unlike the SEMG, single unit analyses indicated that while in some units the MEP size increased significantly, other units appeared to be 'switched off'. This may be explained from the fact that the SEMG is made up of several units, some of which may increase in their MEPs and some may decrease to give an overall average of no change in SEMG data. We have used low-threshold units and while some units increased their MEP amplitudes dramatically, other units that displayed MEPs prior to manipulation showed no MEPs after manipulation, hence it is possible that a similar pattern of changes to MEPs occurs with larger units as well. Our study certainly indicates that spinal manipulation alters the SMU recruitment patterns. As mentioned, this finding of increased low-threshold motor units with spinal manipulation has also been observed in a separate study utilizing the H-reflex methodology [Niazi et al., 2015], where we found a decrease in the H-reflex threshold following spinal manipulation, also suggesting that manipulation selectively alters the excitability of low-threshold motor units in the soleus muscle. Again, these findings have significant implications for clinical application to a variety of patient populations, thus it is critical these findings are examined further.

The CSP has generally been measured from the time of the stimulation to the start of the EMG activity after a period of silence. Our results suggest that this method can be misleading. Similar to what Todd et al (2012), we have shown that using the discharge rate-based method provides a different depiction of both the excitatory and inhibitory events following cortical stimulation. Since this method has been tested directly using known synaptic potentials on regularly discharging motoneurons in rat brain slices, it is reliable to indicate the TMS induced changes in the motoneurone membrane. Using the discharge rate based method, Todd et al. 2012 examined the CSP in an upper limb muscle, first dorsal interosseous (FDI) and suggested that the CSP duration was underestimated in the previous work which used probabilistic methods. We confirm our earlier findings, that to determine the true duration of the CSP one needs to use both the probabilistic and frequency analyses simultaneously and that the most accurate method would be to use of PSTH CUSUM for CSP onset and the PSF CUSUM for CSP endpoint.

Classically defined CSP relies upon reduced motor unit activity as observed in the surface EMG and/or single motor unit prestimulus time histograms (PSTHs). However, as we have shown in regularly discharging motoneurons in rat brain slices, low level of activity does not always indicate inhibitory input to a motoneurone [Türker and Powers, 2005]. *Discharge probability* of a motoneurone may decrease during a falling phase of an excitatory post synaptic potential while the *discharge rate* of these low number of occurrences may actually be higher than the background discharge rate indicating that the excitatory effect is still continuing. In the current study, during the CSP, as indicated by low level of activity in the surface EMG and single unit PSTHs, the discharge rate of the underlying units either did not change or slightly increased (Fig 2-4). Therefore, we caution the investigators regarding the authenticity of the inhibitory events underlying the TMS induced CSP. We found that the significant reduction in the discharge rate was observed only when large numbers of discharges of the units returned after a period of low level of activity (classically defined as the end of cortical silent period however it is indicating delayed inhibition according to our brain slice experiments).

Clinical relevance

The changes observed in the I-waves and CSP duration in this study provide evidence that spinal manipulation can result in a significant increase in the excitability of the motor pathways to low threshold motor units of human tibialis anterior muscle. These finding may reflect the mechanisms of increases in strength shown by other study following spinal manipulation [Christiansen et al., 2018; Niazi et al., 2015]. Based on the current study design it is not possible to make any claims regarding segment-specific spinal manipulation effects. The current study took a pragmatic approach and allowed the chiropractor to manipulate any dysfunctional segments they found (what chiropractors often call vertebral subluxations). In this study each of the subjects received manipulations at multiple levels of the spine (see Table 4). Future studies may wish to look at the effects of only manipulating dysfunctional segments in one spinal area (cervical, thoracic, lumbo/pelvic) vs manipulation in multiple spinal areas. Future studies may also want to see if it matters whether the chiropractor adjust the segments they deem to be dysfunctional vs random spinal manipulation, or specifically manipulating joints that are not considered dysfunctional.

Furthermore, it would be useful if future studies explore whether or not experience and skill level of the manual therapist matters with regards to obtaining these types of neuromuscular effects.

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REFERENCES

Awiszus F, Feistner H, 1993. The relationship between estimates of Ia-EPSP amplitude and conduction velocity in human soleus motoneurons. Experimental brain research 95, 365–70.

Awiszus F, Feistner H, 1994. Quantification of D- and I-wave effects evoked by transcranial magnetic brain stimulation on the tibialis anterior motoneuron pool in man. Experimental brain research 101, 153–8.

Barker A T, Jalinous R, Freeston I L, 1985. Non-invasive magnetic stimulation of human motor cortex. Lancet (London, England) 1, 1106–7.

Bolton P S, Holland C T, 1996. Afferent signalling of vertebral displacement in the neck of the cat. In: Society for Neuroscience, Abstracts. p. 1802.

Bolton P S, Holland C T, 1998. An in vivo method for studying afferent fibre activity from cervical paravertebral tissue during vertebral motion in anaesthetised cats. Journal of neuroscience methods 85, 211–8.

Brasil- Neto J P, Cammarota A, Valls- Solé J, Pascual- Leone A, Hallett M, Cohen L G, Brasil-Neto J P, Cammarota A, Valls-Solé J, Pascual-Leone A, Hallett M, Cohen L G, 1995. Role of intracortical mechanisms in the late part of the silent period to transcranial stimulation of the human motor cortex. Acta Neurologica Scandinavica 92, 383–386.

Brinkworth R S A, Türker K S, 2003. A method for quantifying reflex responses from intra-muscular and surface electromyogram. Journal of Neuroscience Methods 122, 179–193.

Bronfort G, Evans R, Anderson A V, Svendsen K H, Bracha Y, Grimm R H, 2012. Spinal manipulation, medication, or home exercise with advice for acute and subacute neck pain: a randomized trial. Annals of internal medicine 156, 1–10.

Bronfort G, Haas M, Evans R L, Bouter L M, 2004. Efficacy of spinal manipulation and mobilization for low back pain and neck pain: a systematic review and best evidence synthesis. The spine journal : official journal of the North American Spine Society 4, 335–356.

BW H, Herzog W, PJ C, MC M, 1990. Experimental measurement of the force exerted during spinal manipulation using the Thompson Technique. J Manipulative Physiol Ther (JMPT) 13, 448–453.

Cantello R, Gianelli M, Civardi C, Mutani R, 1992. Magnetic brain stimulation: the silent period after the motor evoked potential. Neurology 42, 1951–1959.

Chen R, Corwell B, Hallett M, 1999. Modulation of motor cortex excitability by median nerve and digit stimulation. Experimental Brain Research 129, 77–86.

Cheong J Y, Yoon T S, Lee S J, 2003. Evaluations of inhibitory effect on the motor cortex by cutaneous pain via application of capsaicin. Electromyography and clinical neurophysiology 43, 203–210.

Christiansen T L, Niazi I K, Holt K, Nedergaard R W, Duehr J, Allen K, Marshall P, Türker K S, Hartvigsen J, Haavik H, 2018. The effects of a single session of spinal manipulation on strength and cortical drive in athletes. European journal of applied physiology.

Cooperstein R, Haneline M, Young M, 2010. Interexaminer reliability of thoracic motion palpation using confidence ratings and continuous analysis. Journal of chiropractic medicine 9, 99–106.

Cooperstein R, Young M, Haneline M, 2013. Interexaminer reliability of cervical motion palpation using continuous measures and rater confidence levels. The Journal of the Canadian Chiropractic Association 57, 156–164.

Daligadu J, Haavik H, Yielder P C, Baarbe J, Murphy B, 2013. Alterations in cortical and cerebellar motor processing in subclinical neck pain patients following spinal manipulation. Journal of manipulative and physiological therapeutics 36, 527–537.

Eisenberg E, Chistyakov A V, Yudashkin M, Kaplan B, Hafner H, Feinsod M, 2005. Evidence for cortical hyperexcitability of the affected limb representation area in CRPS: a psychophysical and transcranial magnetic stimulation study. Pain 113, 99–105.

Ellaway P H, 1978. Cumulative sum technique and its application to the analysis of peristimulus time histograms. Electroencephalography and clinical neurophysiology 45, 302–304.

Fryer G, Morris T, Gibbons P, 2004. Paraspinal muscles and intervertebral dysfunction: part one. Journal of manipulative and physiological therapeutics 27, 267–274.

Gross A, Miller J, D'Sylva J, Burnie S J, Goldsmith C H, Graham N, Haines T, Bronfort G, Hoving J L, 2010. Manipulation or mobilisation for neck pain: a Cochrane Review. Manual therapy 15, 315–333.

Haavik-Taylor H, Murphy B, 2007a. Cervical spine manipulation alters sensorimotor integration: a somatosensory evoked potential study. Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology 118, 391–402.

Haavik-Taylor H, Murphy B, 2007b. Transient modulation of intracortical inhibition following spinal manipulation. Chiropractic journal of Australia 37, 106–116.

Haavik H, Murphy B, 2011. Subclinical neck pain and the effects of cervical manipulation on elbow joint position sense. Journal of Manipulative and Physiological Therapeutics 34, 88–97.

Haavik H, Murphy B, 2012. The role of spinal manipulation in addressing disordered sensorimotor integration and altered motor control. Journal of Electromyography and Kinesiology 22, 768–776.

Herzog W, 1996. Mechanical, physiologic, and neuromuscular considerations of chiropractic treatments. Advances in chiropractic 3, 269–285.

Herzog W, Conway P J, Zhang Y T, Gal J, Guimaraes A C, 1995. Reflex responses associated with manipulative treatments on the thoracic spine: a pilot study. Journal of manipulative and physiological therapeutics 18, 233–236.

Hestbaek L, Leboeuf-Yde C, 2000. Are chiropractic tests for the lumbo-pelvic spine reliable and valid? A systematic critical literature review. Journal of manipulative and physiological therapeutics 23, 258–275.

Hubka M J, Phelan S P, 1994. Interexaminer reliability of palpation for cervical spine tenderness. Journal of manipulative and physiological therapeutics 17, 591–595.

Inghilleri M, Berardelli A, Cruccu G, Manfredi M, 1993. Silent period evoked by transcranial stimulation of the human cortex and cervicomedullary junction. The Journal of physiology 466, 521–534.

Jull G, Bogduk N, Marsland A, 1988. The accuracy of manual diagnosis for cervical zygapophysial joint pain syndromes. The Medical journal of Australia 148, 233–236.

Kanovsky P, Bares M, Rektor I, 2003. The selective gating of the N30 cortical component of the somatosensory evoked potentials of median nerve is different in the mesial and dorsolateral frontal cortex: evidence from intracerebral recordings. Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology 114, 981–991.

Kofler M, Fuhr P, Leis A A, Glocker F X, Kronenberg M F, Wissel J, Stetkarova I, 2001. Modulation of upper extremity motor evoked potentials by cutaneous afferents in humans. Clinical neurophysiology 112, 1053–1063.

Kuczynski J J, Schwieterman B, Columber K, Knupp D, Shaub L, Cook C E, 2012. Effectiveness of physical therapist administered spinal manipulation for the treatment of low back pain: a systematic review of the literature. International journal of sports physical therapy 7, 647–662.

Kukowski B, Haug B, 1992. Quantitative evaluation of the silent period, evoked by transcranial magnetic stimulation during sustained muscle contraction, in normal man and in patients with stroke. Electromyography and clinical neurophysiology 32, 373–378.

Lazzaro V Di, Oliviero A, Profice P, Insola A, Mazzone P, Tonali P, Rothwell J C, 1999. Effects of voluntary contraction on descending volleys evoked by transcranial electrical stimulation over the motor cortex hand area in conscious humans. Experimental brain research 124, 525–528.

Lazzaro V Di, Ziemann U, 2013. The contribution of transcranial magnetic stimulation in the functional evaluation of microcircuits in human motor cortex. Frontiers in neural circuits 7, 18.

Loo C K, Taylor J L, Gandevia S C, McDarmont B N, Mitchell P B, Sachdev P S, 2000. Transcranial magnetic stimulation (TMS) in controlled treatment studies: are some “sham” forms active? Biological psychiatry 47, 325–331.

Marshall P, Murphy B, 2006. The effect of sacroiliac joint manipulation on feed-forward activation times of the deep abdominal musculature. Journal of manipulative and physiological therapeutics 29, 196–202.

Mauguiere F, Desmedt J E, Courjon J, 1983. Astereognosis and dissociated loss of frontal or parietal components of somatosensory evoked potentials in hemispheric lesions. Detailed correlations with clinical signs and computerized tomographic scanning. Brain : a journal of neurology 106 (Pt 2), 271–311.

Merton P A, Morton H B, 1980. Stimulation of the cerebral cortex in the intact human subject. Nature 285, 227.

Miles T S, Türker K S, 1986. Does reflex inhibition of motor units follow the “size principle”? Experimental brain research 62, 443–445.

Miles T S, Türker K S, 1987. Decomposition of the human electromyogramme in an inhibitory reflex. Experimental brain research 65, 337–342.

Miles T S, Türker K S, Le T H, 1989. Ia reflexes and EPSPs in human soleus motor neurones. Experimental Brain Research 77, 628–636.

Murphy B A, Dawson N J, Slack J R, 1995. Sacroiliac joint manipulation decreases the H-reflex. Electromyography and clinical neurophysiology 35, 87–94.

Niazi I K, Türker K S, Flavel S, Kinget M, Duehr J, Haavik H, 2015. Changes in H-reflex and V-waves following spinal manipulation. Experimental Brain Research 233, 1165–1173.

Patton H D, Amassian V E, 1954. Single and multiple-unit analysis of cortical stage of pyramidal tract activation. Journal of neurophysiology 17, 345–363.

Pickar J G, Wheeler J D, 2001. Response of muscle proprioceptors to spinal manipulative-like loads in the anesthetized cat. Journal of Manipulative and Physiological Therapeutics 24, 2–11.

Roick H, Giesen H J Von, Benecke R, 1993. On the origin of the postexcitatory inhibition seen after transcranial magnetic brain stimulation in awake human subjects. Experimental brain research 94, 489–498.

Rossi S, della Volpe R, Ginanneschi F, Ulivelli M, Bartalini S, Spidalieri R, Rossi A, 2003. Early somatosensory processing during tonic muscle pain in humans: relation to loss of proprioception and motor “defensive” strategies. Clinical neurophysiology : official journal of the International

Federation of Clinical Neurophysiology 114, 1351–1358.

Rossini P M, Babiloni F, Bernardi G, Cecchi L, Johnson P B, Malentacca A, Stanzione P, Urbano A, 1989. Abnormalities of short-latency somatosensory evoked potentials in parkinsonian patients. Electroencephalography and clinical neurophysiology 74, 277–289.

Rossini P M, Gigli G L, Marciani M G, Zarola F, Caramia M, 1987. Non-invasive evaluation of input-output characteristics of sensorimotor cerebral areas in healthy humans. Electroencephalography and clinical neurophysiology 68, 88–100.

Schmied A, Türker K S, 2001. Periodontal mechanoreceptor input reduces synchronous discharge of voluntarily activated masseter motor units in man. Somatosensory & motor research 18, 141–149.

Siebner H R, Dressnandt J, Auer C, Conrad B, 1998. Continuous intrathecal baclofen infusions induced a marked increase of the transcranially evoked silent period in a patient with generalized dystonia. Muscle & nerve 21, 1209–1212.

Taylor H H, Murphy B, 2008. Altered Sensorimotor Integration With Cervical Spine Manipulation. Journal of Manipulative and Physiological Therapeutics 31, 115–126.

Terao Y, Ugawa Y, Hanajima R, Machii K, Furubayashi T, Mochizuki H, Enomoto H, Shiio Y, Uesugi H, Iwata N K, 2000. Predominant activation of I1-waves from the leg motor area by transcranial magnetic stimulation. Brain research 859, 137–146.

Terao Y, Ugawa Y, Hanajima R, Machii K, Furubayashi T, Mochizuki H, Enomoto H, Shiio Y, Uesugi H, Iwata N K, 2001. A single motor unit recording technique for studying the differential activation of corticospinal volleys by transcranial magnetic stimulation. Brain Research Protocols 7, 61–67.

Tergau F, Wanschura V, Canelo M, Wischer S, Wassermann E M, Ziemann U, Paulus W, 1999. Complete suppression of voluntary motor drive during the silent period after transcranial magnetic stimulation.

Experimental brain research 124, 447–454.

Todd G, Rogasch N C, Türker K S, 2012. Transcranial magnetic stimulation and peristimulus frequencygram. Clinical Neurophysiology 123, 1002–1009.

Türker K S, Cheng H B, 1994. Motor-unit firing frequency can be used for the estimation of synaptic potentials in human motoneurones. Journal of neuroscience methods 53, 225–234.

Türker K S, Powers R K, 2005. Black box revisited: a technique for estimating postsynaptic potentials in neurons. Trends in neurosciences 28, 379–386.

Türker K S, Yang J, Brodin P, 1997. Conditions for excitatory or inhibitory masseteric reflexes elicited by tooth pressure in man. Archives of Oral Biology 42, 121–128.

Waberski T D, Buchner H, Perkuhn M, Gobbele R, Wagner M, Kucker W, Silny J, 1999. N30 and the effect of explorative finger movements: a model of the contribution of the motor cortex to early somatosensory potentials. Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology 110, 1589–1600.

Werhahn K J, Kunesch E, Noachtar S, Benecke R, Classen J, 1999. Differential effects on motorcortical inhibition induced by blockade of GABA uptake in humans. The Journal of physiology 517, 591–597.

Zhu Y, Haldeman S, Hsieh C-Y J, Wu P, Starr A, 2000. Do cerebral potentials to magnetic stimulation of paraspinal muscles reflect changes in palpable muscle spasm, low back pain, and activity scores? Journal of manipulative and physiological therapeutics 23, 458–464.

Zhu Y, Haldeman S, Starr A, Seffinger M A, Su S-H, 1993. Paraspinal muscle evoked cerebral potentials in patients with unilateral low back pain. Spine 18, 1096–1102.

Tables

Table 1: *The characteristics of the cortical silent period (CSP) as measured with three different methods. In the first column, the CSP is analysed from rectified averaged SEMG data from the subjects according to the classical method of considering the onset of CSP from stimulus onset and the endpoint measured from the end of apparent inhibition measured from the SEMG-CUSUM. The middle column calculates the onset and endpoint of the CSP from the SMU PSTH-CUSUM as described in* [Todd et al., 2012] *(n = 21 identical SMUs pre and post spinal manipulation and n = 12 identical SMUs pre and post control). The final column calculates the onset from the PSTH-CUSUM and endpoint of CSP from the PSF-CUSUM in those units with genuine inhibition (n = 7 identical SMUs pre and post spinal manipulation and n = 5 identical SMUs pre and post control)as seen in the PSF-CUSUM as recommended in* [Todd et al., 2012]*. All methods of analysis for CSP resulted in a significant reduction following spinal manipulation with no change following the control intervention.*

		From stimulus onset to SEMG-CUSUM end		***From PSTH-CUSUM onset to end***		***From PSTH-CUSUM onset to PSF-CUSUM end***	
		Mean	***SD***	***Mean***	***SD***	***Mean***	***SD***
Pre SM	*onset*	0	*0*	*46.9*	*11.2*	*46.9*	*11.2*
	duration	121.9	*43.1*	*83.7*	*37.5*	*133.01*	*55.6*
Post SM	*onset*	0	*0*	*44.7*	*7.8*	*44.7*	*7.8*
	duration	102.5**	*33.7*	*58.4***	*33.8*	*98.35**	*44.1*
Pre control	*onset*	0	*0*	*41.6*	*5.4*	*41.6*	*5.4*
	duration	121.3	*37.6*	*62.6*	*32.2*	*131.9*	*45.1*
Post control	*onset*	0	*0*	*42.0*	*7.2*	*42.0*	*7.2*
	duration	136.6	*49.1*	*60.7*	*29.1*	*145.7*	*43.7*

*** $p < 0.01$ compared to pre-intervention value*
** $p < 0.05$ compared to pre-intervention value*

Table 2: *The individual I-wave amplitudes (as a fraction of pre-intervention amplitudes) as measured from the prestimulus CUSUM for those motor units were identical post-intervention and showed genuine excitations in the PSF. This was found in n = 12 (out of total 21 similar) SMUs for the first I-wave pre/post manipulation, and n = 8 (out of total 21 similar) SMUs for the second I-wave pre/post spinal manipulation intervention. For the control intervention, this was found in n = 10 (out of total 12 similar) SMUs for the first I-wave and n = 7 (out of total 12 similar) SMUs for the second I-wave. Note the large increases in I-wave excitation following spinal manipulation.*

		Peristimulus time histogram (PSTH)				***Peristimulus frequencygram (PSF)***			
		I-wave 1		***I wave 2***		***I-wave 1***		***I wave 2***	
		Mean	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***
Pre SM	***Onset***	*31.8*	*4.6*	*42.6*	*4.5*	*34.0*	*4.6*	*42.1*	4.1
	Ampl	*1*	*0*	*1*	*0*	*1*	*0*	*1*	0
Post SM	***Onset***	*31.7*	*4.2*	*40.7*	*4.7*	*33.2*	*4.0*	*39.6*	3.2
	Ampl	*1.9*	*2.4*	*2.8*	*2.8*	*4.3**	*5.5*	*5.3*	8.2
Pre	***Onset***	*31.7*	*2.5*	*37.7*	*5.5*	*33.5*	*4.0*	*40.9*	6.2
control	***Ampl***	*1*	*0*	*1*	*0*	*1*	*0*	*1*	0
Post	***Onset***	*32.0*	*2.8*	*40.0*	*4.4*	*33.7*	*3.6*	*41.5*	5.8
control	***Ampl***	*1.5*	*1.8*	*1.5*	*1.4*	*1.95*	*2.4*	*11.21*	25.14

Pre/post SM identical SMUs; first I wave n=12, second I wave n=8
Pre/post Control identical SMUs; first I wave n=10, second I wave n=7
** p < 0.05 compared to pre-intervention value*

Table 3: *The characteristics of the MEP amplitudes and latencies from SEMG and PSTH data normalised to number of stimuli. From SEMG data the MEP amplitude (in mV) was measured peak to peak from the non-rectified SEMG traces, and the latencies (in ms) was measured from averaged SEMG CUSUM data from all subjects pre/post spinal manipulation and pre/post control. From the PSTH data the MEP amplitudes (in k.ms) and latencies (in ms) were measured from the PSTH-CUSUM from identical units pre/post spinal manipulation and pre/post control. Note there were no significant changes in onset time of MEPs using either method.*

		SEMG		***PSTH CUSUM***	
		Mean	***SD***	***Mean***	***SD***
Pre SM	*onset*	*33.6*	*3.8*	*30.1*	*5.2*
	ampl	*2.3*	*4.1*	*0.4*	*0.4*
Post SM	*onset*	*33.7*	*3.4*	*30.5*	*5.4*
	ampl	*2.2*	*4.1*	*0.4*	*0.3*
Pre control	*onset*	*31.8*	*2.1*	*31.1*	*3.2*
	ampl	*1.3*	*1.9*	*0.4*	*0.2*
Post control	*onset*	*32.3*	*2.1*	*30.6*	*4.2*
	ampl	*1.1*	*1.6*	*0.6*	*0.5*

Table 4: *Spinal manipulation sites for each subject*

Sub ID	***Spinal Manipulations***
1	*Right SI, T9, T4, C7, C2*
2	*Left SI, T12, T1, C1*
3	*Left SI, T10, T8, T4, C3, left Occiput*
4	*right SI, T4, C4, C1*
5	*Left SI, L3, T7, C3, C1*
6	*Left SI, L1, T10, C4, left occiput*
7	*left SI, T10, T5, T1, left occiput, C2*
8	*Left SI, T10, T6, T4, C1, C3*
9	*Left SI, T4, C2, C1*
10	*left SI, T6, C2*
11	*left SI, T9, T4, L3, right occiput, C2*
12	*L & R SI, L2, T11, T7, C4*
13	*L & R SI, T6, T3, C1*
14	*left SI, T7, C2,*
15	*Right SI, T6, C7, C2*
16	*Left SI, T6, Left occiput, C2*
17	*left SI, T10, T6, C1, C4*
18	*Left occiput, C2, T6, T1,*

Figures

Fig 1: *a**)** Raw trace of average SEMG from one subject pre and post spinal manipulation showing almost identical MEPs. b**)** SMU MEP showing a reduction in CSP with the help of a dashed vertical line. Time '0 s' is where the stimulation occurs.*

Fig 2: *Surface EMG and single motor unit data from one subject during threshold transcranial magnetic stimulation delivered during weak ankle dorsiflexion pre and post spinal manipulation (the left column is pre and the right column is post). Several hundred stimuli were delivered (n = 184 (pre) and n=130 (post)). (a) The macro EMG of the unit, showing that it is the same unit both pre and post spinal manipulation. (b) Cumulative sum of peristimulus time histogram (PSTH CUSUM), dashed horizontal lines represent error bars. (c) Peristimulus time histogram (PSTH). (d) Cumulative sum of peristimulus time frequencygram (PSF CUSUM), dashed horizontal lines represent error bars. (e) Peristimulus time frequencygram (PSF). The vertical line represents the timing of TMS. Note that the PSTH of the unit indicates several peaks separated by few milliseconds. In the PSTH CUSUM these separate peaks make separate steps. PSF and its CUSUM indicate further that these PSTH peaks are actually excitatory events as the discharge rate underlying them higher than the prestimulus discharge rate. PSF also indicates that the lower number of spikes during the CSP can actually have a higher discharge rate than the prestimulus values. Furthermore, as illustrated in this figure, the end of the CSP cannot be the timing of the reoccurrence of spikes as it was classically accepted since the discharge rate of these spikes is actually lower than the prestimulus rate indicating that the inhibitory effect is still continuing* [Türker and Powers, 2005].

Fig 3: *Surface EMG and single motor unit data from one subject during threshold transcranial magnetic stimulation delivered during weak ankle dorsiflexion pre and post spinal manipulation (the left column is pre and the right column is post). Several hundred stimuli were delivered (n = 136 (pre) and n=199 (post)). (a) The macro EMG of the unit, showing it is the same unit both pre and post spinal manipulation. (b) Cumulative sum of peristimulus time histogram (PSTH CUSUM), dashed horizontal lines represent error bars. (c) Peristimulus time histogram (PSTH). (d) Cumulative sum of peristimulus time frequencygram (PSF CUSUM), dashed horizontal lines represent error bars. (e) Peristimulus time frequencygram (PSF). The vertical line represents the timing of TMS. Note in B that the down going phase (CSP) is shorter after the manipulation. Also note that during the CSP indicated in panel B, the discharge rate (d and e) does not change indicating that there is no genuine fall in discharge rate during the CSP, hence no genuine inhibition present.*

Fig 4: *Surface EMG and single motor unit data from one subject during threshold transcranial magnetic stimulation delivered during weak ankle dorsiflexion pre (slightly noisy recording) and post the control intervention (the left column is pre and the right column is post). Several hundred stimuli were delivered (n = 210 (pre) and n=131 (post)). (a) The macro EMG of the unit, showing that it is the same unit both pre and post spinal manipulation. (b) Cumulative sum of peristimulus time histogram (PSTH CUSUM), dashed horizontal lines represent error bars. (c) Peristimulus time histogram (PSTH). (d) Cumulative sum of peristimulus time frequencygram (PSF CUSUM), dashed horizontal lines represent error bars. (e) Peristimulus time frequencygram (PSF). The vertical line represents the timing of TMS. Note that the discharge rate of the motor unit does not decrease during the CSP as determined using the SEMG and PSTH. Also note that a genuine reduction in the discharge rate actually occurs when the 'motor units resume their activity' which was classically accepted as the end of CSP duration.*

Fig 5: *PSTHs for the five identical SMUs pre and post manipulation showing the latency of I-waves in the abscissae. Note the increase in all identical units post manipulation*

Fig 6: *PSTHs for one identical SMUs pre and post control showing the latency of I-waves in the abscissae.*

Fig 1:

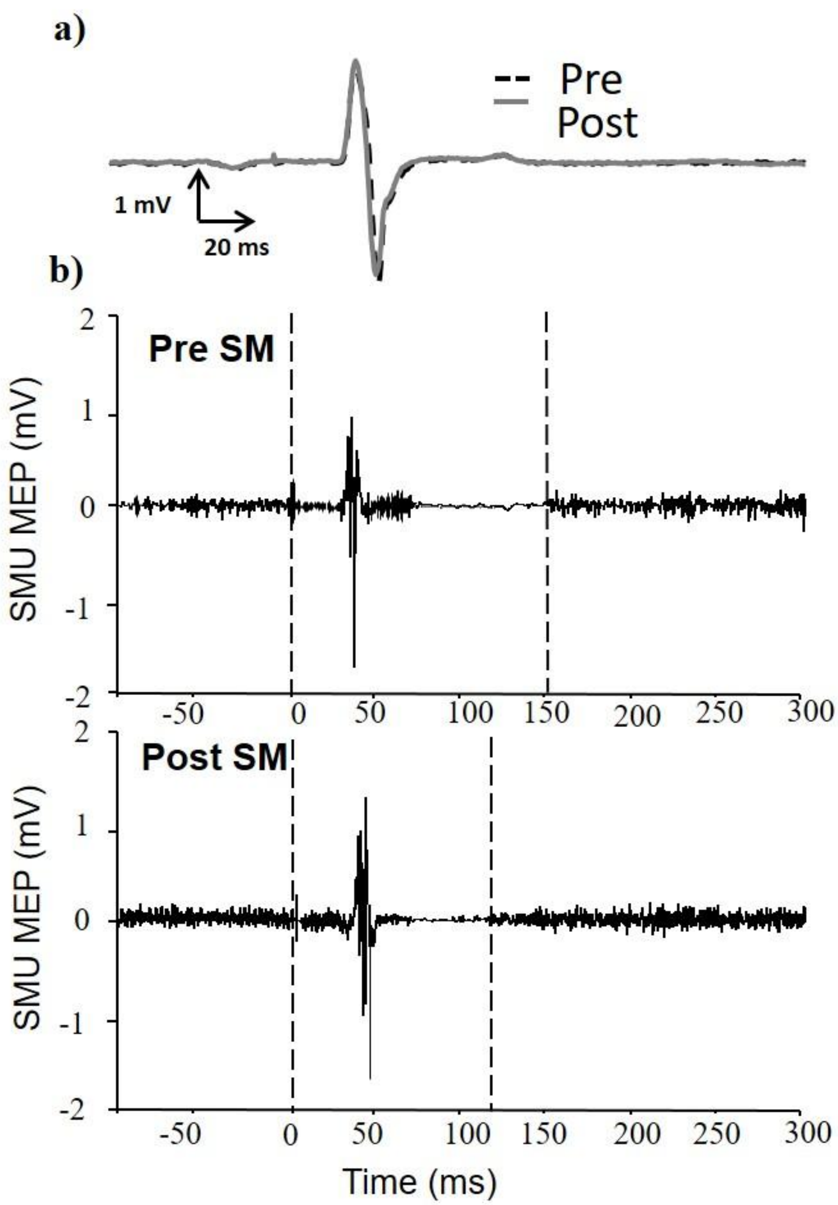

a)
Pre
Post
1 mV
20 ms
b)
Pre SM
SMU MEP (mV)
2
1
0
-1
-2
-50
0
50
100
150
200
250
300
Post SM
SMU MEP (mV)
Time (ms)

Fig 2:

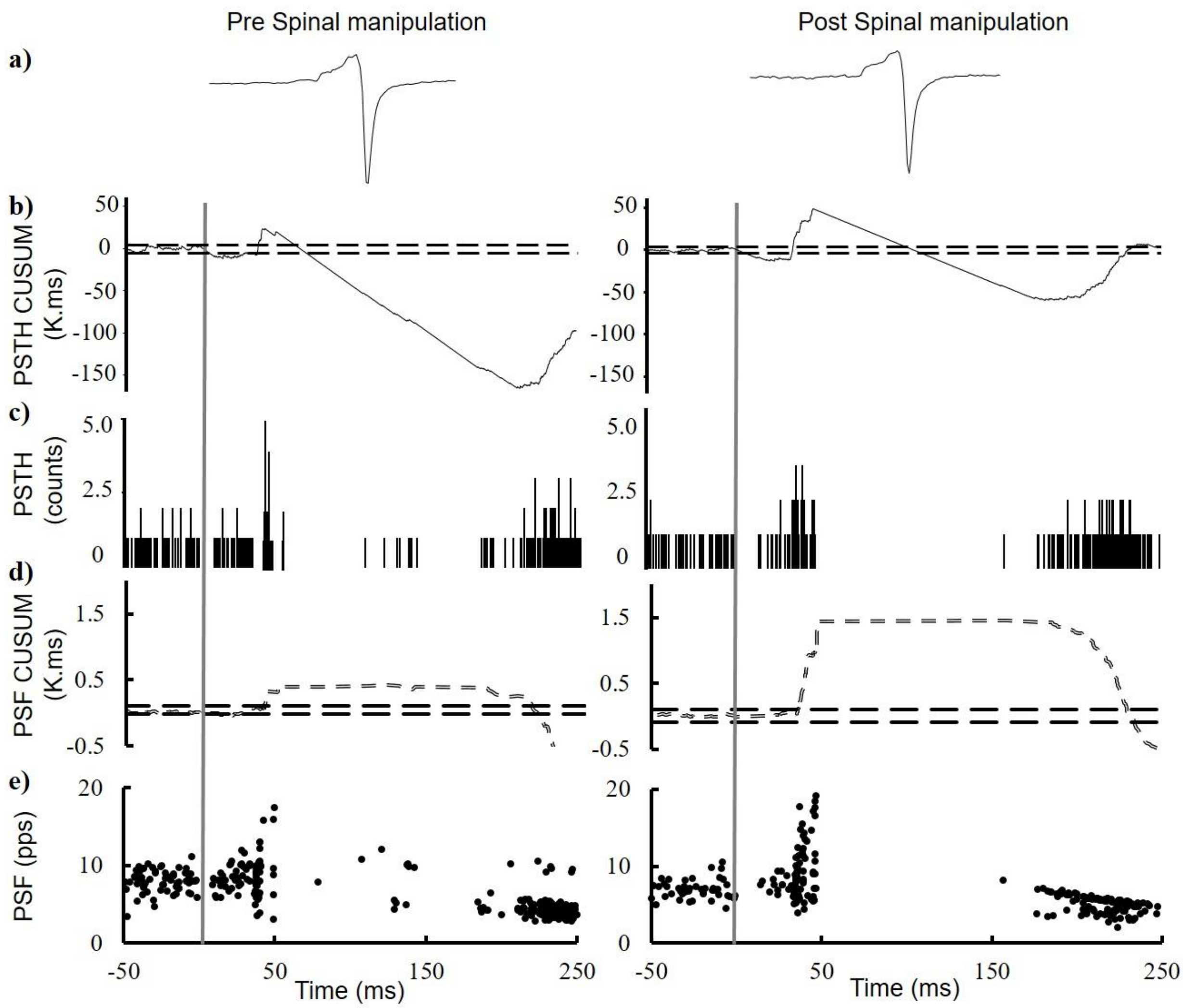

Pre Spinal manipulation
Post Spinal manipulation
a)
b)
PSTH CUSUM (K.ms)
50
0
-50
-100
-150
c)
PSTH (counts)
5.0
2.5
0
d)
PSF CUSUM (K.ms)
1.5
0.5
-0.5
e)
PSF (pps)
20
10
0
-50
50
150
250
Time (ms)

Fig 3:

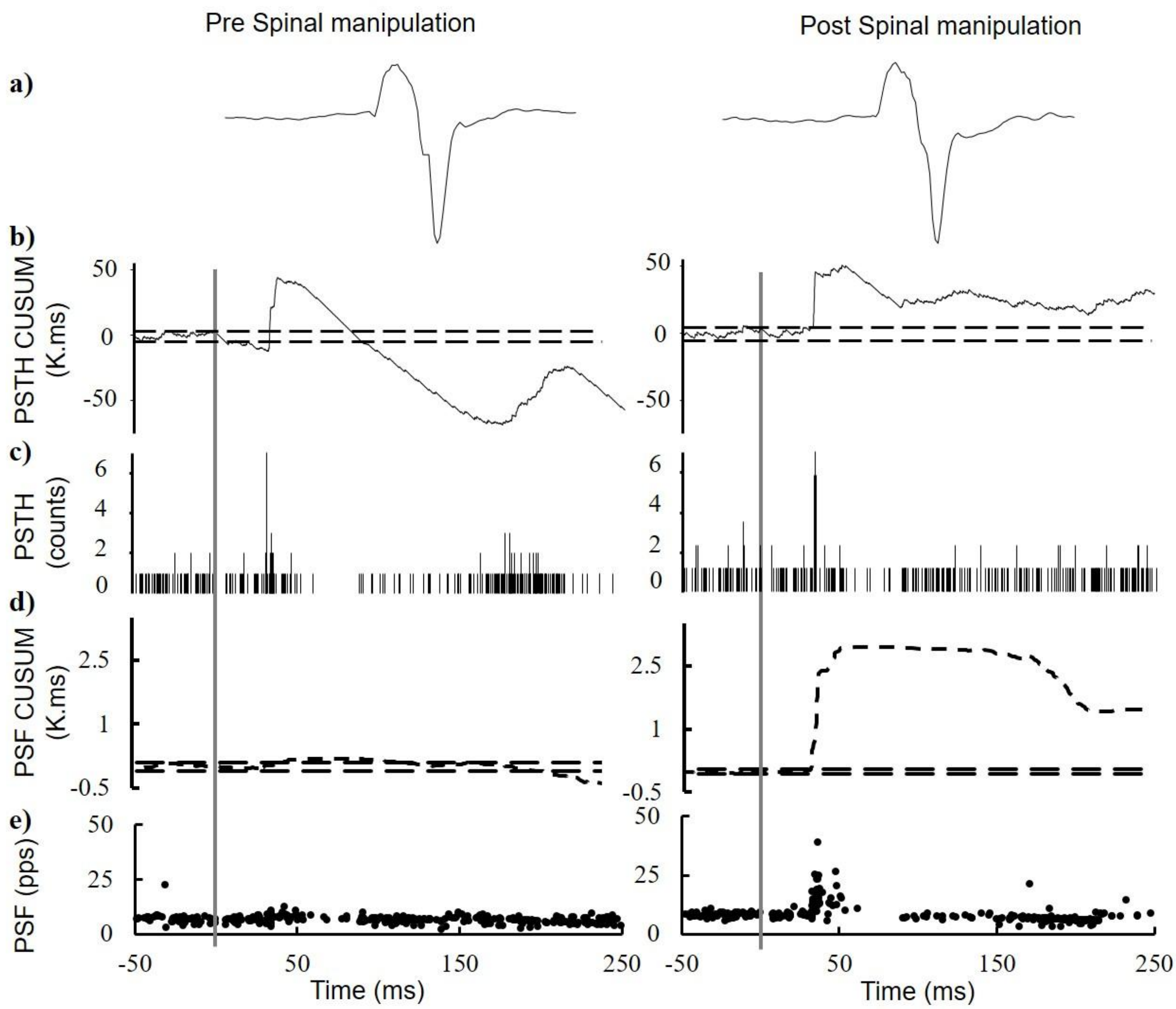

Pre Spinal manipulation
Post Spinal manipulation
a)
b)
PSTH CUSUM (K.ms)
50
0
-50
c)
PSTH (counts)
6
4
2
0
d)
PSF CUSUM (K.ms)
2.5
1
-0.5
e)
PSF (pps)
50
25
0
-50
50
150
250
Time (ms)

Fig 4:

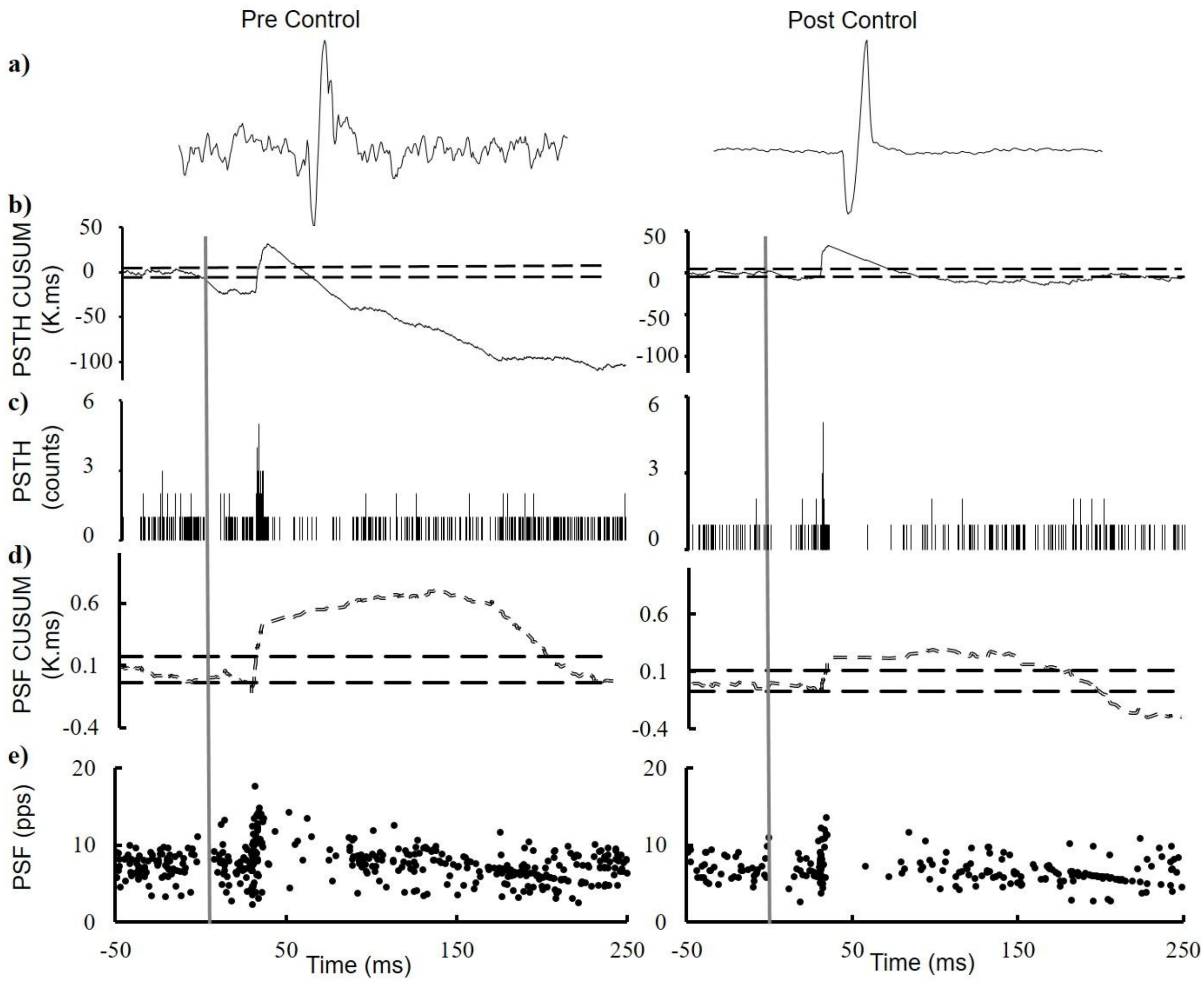

Pre Control
Post Control
a)
b)
PSTH CUSUM (K.ms)
50
0
-50
-100
c)
PSTH (counts)
6
3
0
d)
PSF CUSUM (K.ms)
0.6
0.1
-0.4
e)
PSF (pps)
20
10
0
-50
50
150
250
Time (ms)

Fig 5:

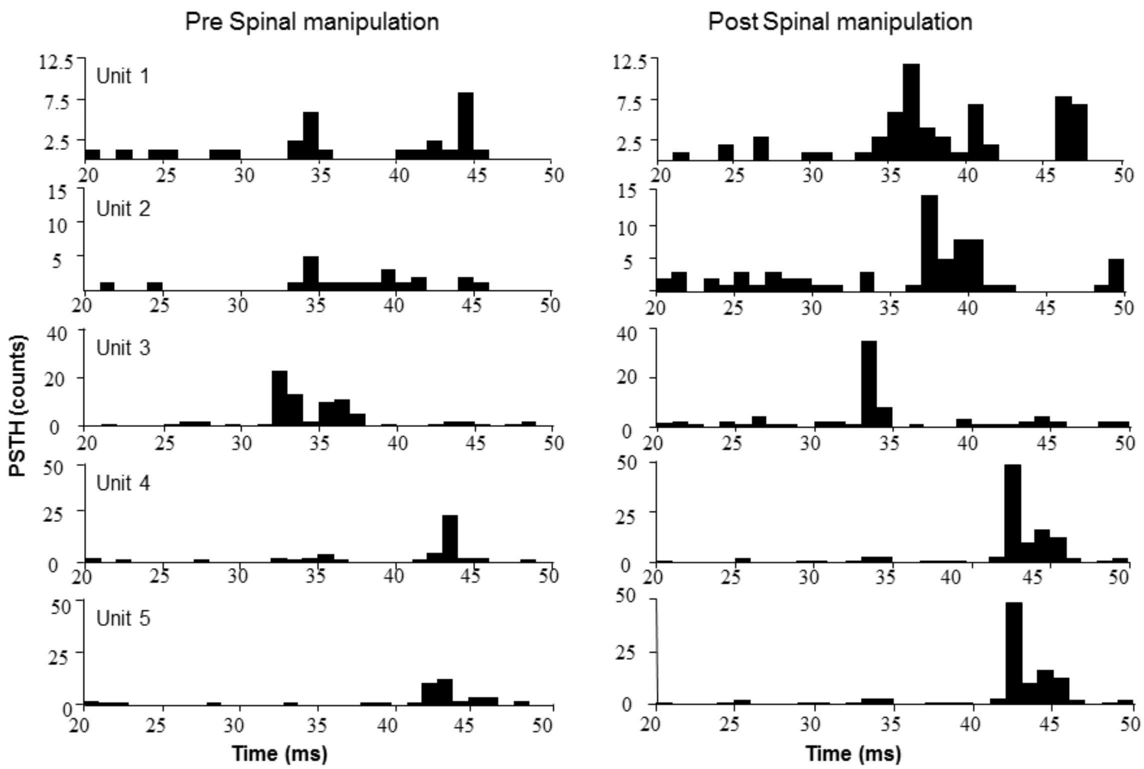
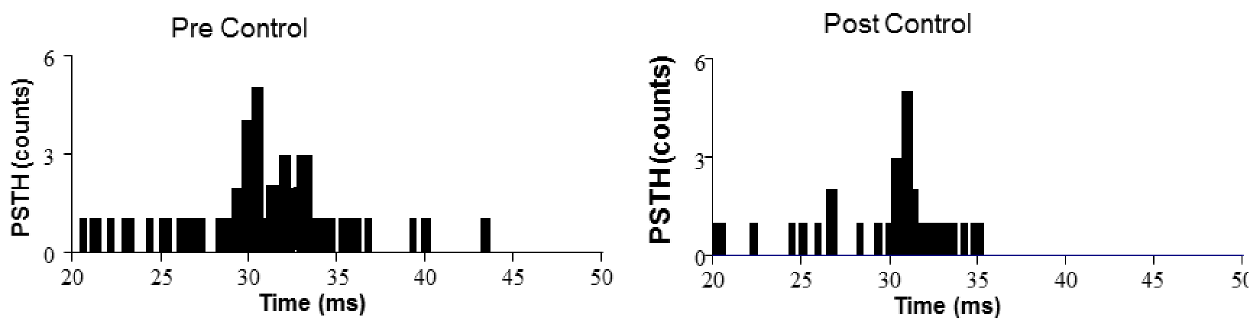

Pre Spinal manipulation
Post Spinal manipulation
Unit 1
Unit 2
Unit 3
Unit 4
Unit 5
PSTH (counts)
Time (ms)
Time (ms)

Fig 6:


Pre Control
PSTH (counts)
6
3
0
20
25
30
35
40
45
50
Time (ms)
Post Control
PSTH (counts)
6
3
0
20
25
30
35
40
45
Time (ms)

Heidi Haavik Dr Heidi Haavik is the Director of Research at the New Zealand College of Chiropractic. She is a chiropractor and has a PhD in human neurophysiology from the University of Auckland. She is the author of 'The Reality Check: A quest to Understand Chiropractic from the inside out'. Dr Haavik has used her neurophysiology expertise to study the neuroplastic mechanisms spinal manipulation. Her current research interests focus on understanding the implications of spinal dysfunction and the effects of chiropractic care on central neural function, health and quality of life.

Imran Khan Niazi received the B.Sc. degree in Electrical engineering (specialization: Biomedical Engineering) from the Riphah International University, Islamabad, Pakistan, in 2005, and the master's in biomedical engineering from University & FH Luebeck, Luebeck, Germany in 2009 and later he got his PhD from Center of sensory motor interaction, Health Science Technology Department, University of Aalborg, Aalborg, Denmark in 2012. After working as postdoc for a year he moved to New Zealand in 2013, where he is currently working as Senior Research Fellow at New Zealand College of Chiropractic. His research interests focus on rehabilitation engineering with the patient-centred approach. He is interested in studying and understanding the altered mechanism of motor control and learning in neurological disorder to develop various technologies that can enhance the QOL of these patients.

Mads Jochumsen received his Bachelor and Master of Science degrees within Biomedical Engineering from Aalborg University, Denmark. He did his PhD at the Doctoral School of Medicine at Aalborg University within the area of brain-computer interfacing for neurorehabilitation where he currently holds a position as Assistant Professor. His research interests include signal processing of electrophysiological signals and neuroplasticity for rehabilitation.

Paulius Uginčius is a qualified prosthodontist and in 2015 was awarded his PhD degree in Human Masticatory Control (supervised by Prof Kemal S. Türker) at Institute of Physiology and Pharmacology, Lithuanian University of Health Sciences, Kaunas, Lithuania. He has more than ten years of dental and medical student training experience at the Institute of Physiology and Pharmacology, Lithuanian University of Health Sciences, Lithuania.

From 2007 Paulius started visiting Prof Kemal Türker's Neurophysiology lab in Turkey and since then together with his research team they have contributed to the knowledge of how human masticatory muscles are synaptically connected with the periodontal mechanoreceptors, muscle spindles and mucosal afferents within the lips.

Oguz Sebik was born in Izmir, Turkey. He received his bachelor's degree in Physics in 1998 from Koç University and his master's degree in Psychology in 2000 from University of New Haven. He received his Ph.D. from Ege University in Biophysics in 2012. He was employed as a post-doctoral researcher at Koç University, School of Medicine until he left in June 2016. Since then, he has been enjoying the life out of academia.

Gizem Yilmaz was born in Izmir, Turkey. She received her bachelor's degree in Bioengineering in 2012 from Ege University and her master's degree in Medical Physiology in 2014 from Koç University. She is currently a PhD student in Biomedical Sciences and Engineering in Koç University. Her PhD studies include the investigation of the neuronal pathways involved in motor control and the relationship between EEG and EMG signals.

Muhammad Samran Navid received the B.Sc degree in computer engineering from NUCES-FAST, Lahore, Pakistan in 2012, and the M.Sc degree in biomedical engineering from NUST, Islamabad, Pakistan in 2015. He started his Ph.D. at Aalborg University, Aalborg, Denmark in 2016. He is a research associate at New Zealand College of Chiropractic, Auckland, New Zealand since 2017. His current research interests are focused on the effect of chiropractic care on brain activity; BCI for neurorehabilitation and signal processing of electroencephalography.

Mustafa Görkem Özyurt received his bachelor's degree in Bioengineering Department from Ege University, Turkey with a high honors degree as the top-ranked student in 2014. He worked in cellular neuroscience using three-dimensional tissue culture models. In 2013, he visited Magdeburg, Germany as an Erasmus intern to investigate neuronal differentiation in Genetics and Molecular Neurobiology Department. Since 2014, he is studying neuronal circuits using electrical stimulation of the peripheral nerves, transcranial magnetic stimulation of the motor cortex and the response of the single motor units, for his Ph.D. degree in Biomedical Sciences and Engineering in Koç University, Turkey. His thesis is about investigating Renshaw interneurons using electrical stimulation of the motor neurons in human and using optogenetics in the mouse.

Kemal S. Türker is a dentist and obtained his Ph.D. degree in Neurophysiology from the Institute of Physiology, Glasgow University, Scotland. He took up a research position in School of Medicine, Adelaide University, Australia where he worked from 1983 to 2007. He has then returned to his home country, Turkey as the Marie Curie Chair of the European Union. Kemal has devoted all of his research efforts in understanding the synaptic inputs from the brain and peripheral receptors to motoneurons that innervate human muscles. Current projects vary from control of human muscles by the brain and peripheral receptors to modulation of various reflexes during movement.